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6c ADDRESS (City, State, and ZIP Code)
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7b ADDRESS (City, State, and ZIP Code)
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The work described here is part of an ongoing set of studies aimed at characterizing the physiological actions and anatomical organization of the monoaminergic projection systems to the rat cerebral cortex, cerebellum and hypothalamus. The underlying themeof this work is that the endogenous monoamines, norepinephrine (NE) and serotonin (5-HT), serve to modulate central neuronal responsiveness to afferent synaptic inputs and by so doing participate in the cognitive process of selective attention. Specifically, individual studies describe: 1) the effects of NE and 5-HT on rat visual and somatosensory cortical neuron responses to afferent pathway stimulation, 2) topographic organization of the neocortical projection neurons in the serotonergic dorsal raphe nucleus, 3) pharmacological characterization of NE effects in rat lateral hypothalamus and 4) similarity between the modulatory actions of NE and stimulant drugs, cocaine and amphetamine. Overall, the data provide further support for the contention that the diffusely distributed monoamine systems of the mammalian brain may enhance the performance of target neuronal circuits as a function of changing behavioral conditions.

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22a NAME OF RESPONSIBLE INDIVIDUAL
Dr. William O. Berry

22b TELEPHONE (Include Area Code)
(202) 767-5021

22c OFFICE SYMBOL
NL

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FINAL TECHNICAL REPORT

AFOSR-87-0138
"The Role of Central Monoaminergic Systems
in Arousal and Selective Attention."
2-1-87 to 3-31-88

Barry D. Waterhouse, Ph.D.
Department of Physiology and Biophysics
Hahnemann University
Broad and Vine
Philadelphia, PA 19102

1. Summary

The major goal of ongoing studies has been to evaluate the role of the endogenous monoamines, norepinephrine (NE) and serotonin (5-HT), in the transfer of sensory information through neuronal networks of mammalian brain. Previously demonstrated modulatory actions of NE and 5-HT on single cell responses to synaptic inputs in the rat cerebral cortex, cerebellum and hypothalamus have suggested that these monoaminergic systems might enhance neuronal circuit function and participate in the cognitive process of selective attention. Individual studies conducted during the past year have focused on further electrophysiological characterization of the parameters of cell responsiveness regulated by NE and 5-HT as well as identification of the receptor systems responsible for mediating these modulatory effects. Additional studies have revealed a functionally significant topographic organization within monoamine-containing nuclei as well as similarity between the physiological actions of NE and a stimulant drug, i.e. cocaine, which is known to produce alerting and enhanced performance effects in laboratory animals and man. Overall, the data collected during this period of the project provide further support for the contention that the diffusely distributed monoamine systems of the mammalian brain may enhance the performance of target neuronal circuits as a function of changing behavioral conditions. Procedures and apparatus for microiontophoresis and recording in the awake, behaving rat have also been further developed over the past year and are ready to be incorporated into the design of future experiments. Such capabilities will permit more direct tests of monoar ine actions in local circuits of behaving animals.

2. Research Objectives

The primary focus of the project has not deviated from the originally stated aims listed below.

Aim 1. Investigate the basic physiological actions of the noradrenergic and serotonergic projection systems in primary sensory areas of the mammalian brain.

Initial studies will employ iontophoretic methods of drug application to characterize the elemental effects of NE and 5-HT on somatosensory or visual cortical neuronal responses to peripheral stimulation of afferent synaptic pathways or to microiontophoresis of putative transmitter substances. In other studies, stimulation of the locus coeruleus or dorsal raphe nucleus will be employed to cause release of endogenous monoamines at anatomically relevant sites in target neuronal circuits and confirm results observed with iontophoretic application of NE and 5-HT. Similar studies will be carried out in other regions of the brain that relay sensory information and receive monoaminergic projections, e.g. lateral geniculate nucleus, superior colliculus. The goal here will be to further

develop the concept that NE and 5-HT operate in a neuromodulatory mode as part of a signal "gating" or "filtering" mechanism in primary sensory neocortex and other sensory information relay circuits in the brain. While these initial studies will be carried out in anesthetized rats, a major effort will be mounted to examine these issues in awake, behaving animals using recently developed techniques for chronic unit recording and iontophoretic drug application (West and Woodward, 1983).

Aim 2. Analysis of the physiological actions of amphetamine and cocaine at the synaptic level in cerebrocortical and cerebellar circuits. The primary issues to be investigated here are whether these psychostimulant agents can mimic the facilitating actions of NE on neuronal responsiveness to synaptic inputs and putative transmitter substances and whether or not such effects correlate with the overt behavioral responses which have been reported for these drugs. The proposed experiments will employ the electrophysiological assays developed previously for study of the NE system to determine the effects of these drugs on synaptic mechanisms. The merit of this approach derives from the fact that a common set of experimental neurophysiological tests can be used to examine the function of the noradrenergic system (see Aim 1) as well as drugs which are known to interact with this system.

Aim 3. Examine the anatomical organization of monoamine-containing projection neurons with respect to sensory-specific target regions of the CNS. These investigations will employ single and double retrograde tracer techniques and computer-assisted image analysis to study the distribution of monoamine-containing projection neurons with respect to sensory-modality specific target regions in the CNS. Initial studies using retrograde transport of HRP suggest that the monoamine nuclei have an internal organization such that activity in subsets of dorsal raphe and locus coeruleus cells may independently influence separate populations of neurons within serotonergic and noradrenergic terminal fields of the neocortex. Moreover, double-labeling protocols have revealed single dorsal raphe neurons which project to both rat visual cortex and cerebellar paraflocculus, areas which are known to receive visual information. The emphasis of the proposed studies will be to explore the possibility that central monoaminergic projections are organized according to the sensory function of target neuronal circuits and whether such an organization would be consistent with a postulated role of these systems in attentional mechanisms.

3. Status of Research

A brief description of individual studies (see Publications Supported) is provided below.

NE Actions in Lateral Hypothalamus (LH) (Ref. #1,2)

Two initial studies aimed at characterizing NE actions in LH have been completed. One major result of this work was that although NE was found to augment inhibitory responses of LH neurons to GABA in both intact anesthetized rat and tissue slices, there was a clear reduction in the percentage of cells demonstrating this effect in vitro despite normal sensitivity of in vitro cells to the direct depressant actions of NE and GABA. These findings suggest that the mechanism(s) responsible for mediating NE modulatory actions may be selectively compromised in otherwise normally functioning tissue slice preparations. Thus, while the brain tissue slice appears to be a useful model for further study of noradrenergic modulatory phenomena, these investigations emphasize the need for cross-validation of results obtained under in vitro and in vivo conditions. It is also worth noting that implementation of the in vitro technique in our laboratory through these studies, has provided us with a foundation for future intracellular recording experiments directed at mechanistic questions concerning the substrates underlying NE effects on GABA-mediated inhibition.

An additional in vivo study in LH was initiated to examine monoamine influences on LH neuron responses to physiologically significant afferent signals and acetylcholine application. A major new finding of this investigation is that otherwise subthreshold iontophoretic doses of acetylcholine can markedly increase neuronal firing rate during local administration of NE. Likewise, NE can reveal unit responses to physiological stimuli, i.e. portal vein infusion of NaCl or glucose that are otherwise undetectable. These findings suggest that in target areas of the CNS, NE may not only regulate the magnitude of cell responses, but may also influence the threshold of neuronal excitability to afferent signals. Future studies will focus on clarifying such "gating" effects on threshold vs. subthreshold stimuli.

Gating Actions of NE (Ref. #3

Many previous studies have examined the effects of norepinephrine (NE) on neuronal responsiveness to synaptic inputs and putative transmitter substances and have described differential depressant actions of NE on stimulus evoked versus spontaneous discharge such that the "signal to noise" ratio of threshold responses was increased. Similar experimental strategies employing a combination of microiontophoresis, single unit recording and afferent pathway stimulation in intact anesthetized and brain tissue slice preparations have revealed noradrenergic "gating" actions whereby weak or subthreshold synaptic stimuli can evoke threshold neuronal responses in the presence of iontophoretically applied NE or following electrical

stimulation of the locus coeruleus. Overall, these results suggest that potentially threshold excitatory and inhibitory synaptic inputs may normally arrive at central neurons but appear weak or absent except during behavioral conditions favoring the synaptic release of NE. As such, these findings provide evidence that enhancement of signal to noise ratio may not be the only potential modulatory action expressed by NE in noradrenergic target circuits of the mammalian brain.

Norepinephrine and Serotonin Actions in Rat Visual Cortex (Ref. #M1)

A study examining simple and complex cells in rat visual cortex (area 17), demonstrated that NE could enhance and 5HT could suppress multiphasic responses of these neurons to moving visual stimuli. In addition to simple changes in magnitude of response, iontophoretic NE and 5HT produced alterations in phasic, movement evoked discharges such that neuronal discrimination of receptive field borders was enhanced (NE) or suppressed (5HT). Likewise, NE and 5HT altered the direction selectivity of visual cells. These findings suggest that the noradrenergic and serotonergic systems may operate in concert to fine tune the feature extraction properties of the visual cortical circuitry. A particularly interesting noradrenergic effect which should be noted was that in some cases NE revealed responses to visual stimuli which were not observed during the control condition. The implication of this finding is that potentially threshold synaptic inputs may normally arrive at visual cortical neurons but appear absent or extremely weak unless facilitated by NE. Overall, studies in the visual cortex may prove to be the most exciting since they may distinguish the most subtle modulatory influences of the monoamines on sensory information transfer through neuronal networks.

Neuropeptide Actions in the Somatosensory Cortex (Ref. #M2)

A study was recently initiated to investigate the interactions that might occur between transmitter-induced responses of somatosensory neurons and iontophoresis of NE, VIP and SRIF. In a majority of cases, VIP enhanced neuronal responsiveness to transmitter application in a manner similar to that observed with NE. In some cells NE + VIP produced an actual synergistic effect on cortical cell responsiveness. SRIF-induced modulatory effects were either facilitory or antagonistic. Overall, these preliminary observations provide electrophysiological evidence for an interactive modulatory relationship between neocortical peptidergic neurons and noradrenergic cortical afferents. Because of their perpendicular orientation to one another, NE axons and peptide-containing neurons could exert convergent influences on cortical neuronal

responses to afferent inputs such that synergistic or antagonistic modulatory actions are anatomically "focused" within the cortex. Additional studies are needed to further explore this hypothesis, but the initial implication is that endogenous peptides may also have a role in regulating neuronal excitability and synaptic transmission within the cortex. Such modulatory effects could be further characterized using our established protocols and analysis procedures.

Mediation of NE Modulatory Effects by Second Messenger Systems (Ref. #A2,A3,A4)

Several investigations aimed at identifying cellular mechanisms associated with noradrenergic facilitating actions are in progress. One in vivo study has demonstrated that a membrane permeant analog of 3', 5' cyclic AMP can mimic the previously observed facilitating action of NE on GABA responses of Purkinje neurons. Other preliminary experiments conducted in cerebellar tissue slice preparations have confirmed this result and also shown that agents which increase intracellular levels of cyclic AMP, i.e. forskolin - (direct adenyl cyclase activation) and IBMX (phophodiesterase inhibition), can also augment GABA responses of Purkinje neurons. These results provide further evidence that NE augmentation of GABA efficacy in neuronal circuits may be mediated by activation of a beta type adrenergic receptor and subsequently increased levels of intracellular cyclic AMP. Additional in vitro studies in cerebrocortical tissue slices have demonstrated that the phosphotide inositol second messenger system is involved in mediating NE-induced facilitation of excitatory synaptic responses in neocortex. These types of investigations are particularly exciting since they hold the promise of identifying the signal transduction mechanism(s) responsible for mediating the previously observed effects of both NE and 5HT on neuronal responsiveness.

Psychostimulant Drug Studies (Ref. #A1,A6)

A number of studies were initiated to investigate the actions of the psychostimulant compound, cocaine, which is known to interact with noradrenergic synapses. Essentially, the same electrophysiological assays used to define monoamine function in local neuronal circuits were employed to evaluate cocaine actions at central synapses. Using the cerebellar Purkinje cell as a model, we have observed that systemically and locally applied cocaine can augment inhibitory neuronal responses to microiontophoretically applied GABA. Cocaine has also been observed to enhance synaptically-evoked and glutamate-induced excitatory discharges in cerebrocortical neurons. Presumably, these observed effects result indirectly from cocaine actions on NE containing terminals. Nevertheless, further studies are in

progress to complete this investigation and clarify the mode of interaction between exogenously applied cocaine and the endogenous noradrenergic system. The hope is that common modes of action will be observed for cocaine and NE and that some of these effects may be correlated with behavioral actions of these compounds.

Drug Studies in Awake Behaving Animals (Ref. #A5)

A recently initiated study in awake behaving rats is examining the activity of single somatosensory or VPL thalamic neurons, recorded one at a time or simultaneously (up to 5 cells), before and after i.p. injection of cocaine. The initial results indicate that stimulus-induced responses of these cells may be differentially affected by a single dose of cocaine such that in some neurons evoked responses were facilitated whereas responses in others were unchanged or suppressed. Moreover, through spike triggered averaging techniques it has been possible to examine synaptic interactions between cells and demonstrate that cocaine was capable of altering the routing of sensory information within a many neuron ensemble. Aside from the direct implications of this data for local circuit actions of cocaine, these studies demonstrate that single neurons can be recorded one at a time or simultaneously from awake behaving animals. Studies in awake behaving animals will continue and it is anticipated that this approach will provide significant new insights into monoamine and state dependent influences on neuronal excitability in cortical circuits.

Comment on Progress:

During the past funding period, several previously initiated studies have been completed and the manuscripts detailing those results are being prepared for publication. For the most part, these studies have further clarified the lowest threshold actions of NE and 5-HT in cerebral cortex and begun to identify candidate second messenger systems associated with monoamine modulatory effects.

In the coming years we will continue to work toward the completion of many of the ongoing studies described above. In addition, several new protocols will be initiated to further clarify the physiological function of central monoaminergic systems. Specifically, the "gating" actions of NE will be investigated as well as the pharmacology of monoamine modulatory effects in terms of receptor subtypes and transmitter analogues. One experimental strategy which will continue is the interaction of locus coeruleus or dorsal raphe conditioning stimuli with somatosensory or visual cortical neuronal responses to synaptic inputs. In recent studies, thalamo-cortical pathway stimulation has been paired with LC stimulation. The results obtained to

date confirm a previous preliminary finding that activation of the noradrenergic pathway from the locus coeruleus can facilitate cortical neuronal responsiveness to peripheral afferent pathway stimulation; presumably via synaptic release of endogenous NE. A similar investigation of the effects of dorsal raphe (serotonergic) stimulation on cortical neuronal responsiveness is planned.

Studies of the effects of the psychostimulant compound cocaine will also continue. The goal of these studies is to determine if cocaine can mimic the previously characterized facilitating actions of norepinephrine on single unit responsiveness.

Another ongoing project involves the in vitro tissue slice preparation. One experimental strategy which will be a particular focus of our attention is the pharmacological characterization of norepinephrine-induced modulatory actions in cerebrocortical and cerebellar slices. A number of adrenoceptor specific agents (timolol, sotalol, phentolamine) and membrane permeable intracellularly acting compounds (phorbol esters, protein kinase inhibitors, forskolin, IBMX) will be tested with the aim of identifying the cascade of intracellular ("second messenger system") events which mediate NE potentiation of GABA responses.

One additional goal for the next funding period will be the initiation of studies in awake behaving rats. These experiments will examine somatosensory cortical neuronal responsiveness to forepaw stimulation during a variety of controlled behavioral situations, drug states and monoamine pathway stimulation. The goal of these experiments is to identify state dependent changes in sensory transmission that may be associated with activity in central monoaminergic pathways.

4. Publications Supported by AFOSR-87-0138

Papers:

1. Sessler, F.M., Cheng, J-T. and Waterhouse, B.D. 1988. Electrophysiological actions of norepinephrine in rat lateral hypothalamus: I. Norepinephrine induced modulation of LH neuronal responsiveness to afferent synaptic inputs and putative neurotransmitters. Brain Res. 446:77-89.

2. Cheng, J-T., Sessler, F.M., Azizi, S.A., Chapin, J.K. and Waterhouse, B.D. 1988. Electrophysiological actions of norepinephrine in rat lateral hypothalamus: II. An in vitro study of the effects of iontophoretically applied norepinephrine on LH neuronal response to gamma-aminobutyric acid (GABA). Brain Res. 446:90-105.

3. Waterhouse, B.D., Sessler, F.M., Cheng, J-T., Woodward, D.J., Azizi, S.A. and Moises, H.C. 1988. New evidence for a gating action of norepinephrine in central neuronal circuits of mammalian brain. Brain Res. Bull. (In Press).

Manuscripts:

M1. Waterhouse, B.D., Azizi, S.A., Burne, R.A. and Woodward, D.J. Modulation of area 17 simple and complex cell responses to moving visual stimuli during norepinephrine and serotonin microiontophoresis. (In Preparation).

M2. Sessler, F.M. and Waterhouse, B.D. Electrophysiological assessment of synergistic interactions between norepinephrine and VIP in rat cerebral cortex.

Abstracts:

A1. Jimenez-Rivera, C.A., Garcia, E. and Waterhouse, B.D. 1987. Physiology of abuse potential substances in central neuronal circuits: cocaine effects on synaptic transmission of afferent signals to rat somatosensory cortical neurons. Soc. Neurosci. ABst. 13:407.

A2. Waterhouse, B.D., Sessler, F.M., Cheng, J-T. and Yeh, H.H. 1987. Noradrenergic potentiation of cerebellar Purkinje cell responses to GABA: evidence for mediation by an intracellular second messenger system. Soc. Neurosci. Abst. 13:1347.

A3. Waterhouse, B.D., Sessler, F.M. and Mouradian, R.D. 1988. Noradrenergic potentiation of cerebellar Purkinje cell responses to GABA: Pharmacologic specificity with respect to adenosine actions and GABA receptor subtypes. Soc. Neurosci. ABst.

A4. Mouradian, R.D., Sessler, F.M. and Waterhouse. B.D. 1988. Noradrenergic potentiation of excitatory transmitter action in cerebrocortical neurons: Evidence for mediation by alpha receptor linked activation of protein kinase. C. Soc. Neurosci. Abst.

A5. Shin, C-H., Jimenez-Rivera, C., Jin, B.K., Waterhouse, B.D. and Chapin, J.K. 1988. Cocaine alters sensory responsiveness and functional connections within networks of simultaneously recorded neurons in the SI cortex and VPL thalamus of behaving rats. Soc. Neurosci. Abst.

A6. Jimenez-Rivera, C.A. and Waterhouse, B.D. 1988. Physiology of abuse potential substances in central neuronal circuits: Cocaine effects on somatosensory and cerebellar neuronal responses to iontophoretically applied glutamate. Soc. Neurosci. Abst.

5. Professional Personnel directly involved in AFOSR-85-0155

(P.I.) Barry D. Waterhouse, Ph.D.
Associate Professor of Physiology and Biophysics

Francis M. Sessler, Ph.D.
Research Assistant Professor

Carlos A. Jimenez-Rivera, Ph.D.
Research Instructor

6. Coupling Activities

AFOSR - Review of Air Force Sponsored Basic Research in Neuroscience
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"The Role of Central Monoaminergic Systems in Arousal and Selective Attention."

Summary presentation of data to AFOSR Life Sciences Directorate and other research personnel from DoD and AFOSR supported laboratories

7. New discoveries, inventions, etc.

Not applicable